DATA EVALUATION RECORD

NAPHTHALENE

Study Type: Non-guideline; *In Vitro* Dermal Absorption Rate Testing with Human Skin

Work Assignment No. 4-01-142 (MRID 47104501)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; *In Vitro* Dermal Absorption Rate Testing with Human Skin; OPPTS None; OECD 428.

PC CODE: 005801 **DP BARCODE:** D339331
TXR#: 0054592

TEST MATERIAL (RADIOCHEMICAL PURITY): Naphthalene (99.1%)

SYNONYMS: None

CITATION: Tedesco, J. A. (2006) Naphthalene: *in vitro* dermal absorption rate testing. E.I. du Pont de Nemours and Company, Haskell[SM] Laboratory for Health and Environmental Sciences, Newark, DE. Laboratory Project ID: DuPont-17707, March 7, 2006. MRID 47104501. Unpublished

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EXECUTIVE SUMMARY: The purpose of this study was to determine a permeability coefficient (Kp) and short-term penetration rate for naphthalene using human cadaver skin mounted in an *in vitro* diffusion cell. These data were obtained to satisfy the rule "*In Vitro* Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Safety and Health Administration," published in the Federal Register on April 26, 2004 (Vol. 69, No. 80). In this non-guideline *in vitro* dermal penetration study (MRID 47104501), [UL-^{14}C]naphthalene (99.1% radiochemical purity; Lot No. 068H9600/01) was applied to human epidermal skin membranes. Skin membranes were prepared from the abdominal donor skin of three human males, and mounted in diffusion cells. The exposed skin area was 0.64 cm^2. Membrane integrity was determined by electrical impedance. For the Kp experiment, the test compound was applied at a dose volume of 100 µL/cm^2 to a total of six skin membranes. Receptor fluid samples were taken at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h post-dosing. For the short-term penetration rate experiments, the test compound was applied at a dose volume of 30 µL/cm^2 to a total of 12 skin membranes. The exposures were terminated at either 10 or 60 minutes post-dosing, with equal numbers of membranes taken for each time point.

The integrity of the skin membranes was not affected by exposure to the test substance. The test substance penetrated the skin readily. The steady-state penetration determined from a minimum of four data points was 12.3 µg equivalents/cm^2/h. The permeability coefficient was calculated to be 1.38x10^{-4} cm/h. Following a 10 minute exposure, a penetration rate of 142.6 µg

equivalents/cm^2/h was calculated. Following a 60 minute exposure, a penetration rate of 25.0 µg equivalents/cm^2/h was calculated.

This study is **acceptable/non-guideline**.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

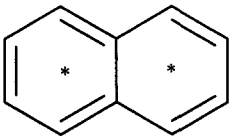
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Radiolabelled test material: [UL-^{14}C]Naphthalene

Radiochemical purity:	99.1% (HPLC)
Specific activity:	Not provided
Lot no:	068H9600/01
Source:	Sigma-Aldrich (St. Louis, MO)
Structure	



(position of ^{14}C-label indicated by *)

Non-radiolabelled test material: Naphthalene

Description:	White crystalline flakes
Lot no:	09424LC
Source:	Sigma-Aldrich (St. Louis, MO)
Purity:	99.8%
CAS # of TGAI:	91-20-3

2. **Receptor fluid:** 0.9% saline fortified with 6% polyethoxyoleate (polyethylene glycol 20 oleyl ether)

3. **Purpose of study:** The purpose of this study was to determine a permeability coefficient (Kp) and short-term penetration rate for naphthalene using human cadaver skin mounted in an *in vitro* diffusion cell. These data were obtained to satisfy the rule "*In Vitro* Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Safety and Health Administration," published in the Federal Register on April 26, 2004 (Vol. 69, No. 80).

B. STUDY DESIGN

1. **Dose formulation:** The radiolabelled and non-radiolabelled test substances were mixed with isopropyl myristate to achieve a target concentration of 100 mg/mL. Homogeneity and specific activity of the formulation were determined by liquid scintillation counting (LSC). Additionally, the maximum concentration of the test formulation that was soluble in the receptor fluid was determined prior to initiation of the study. It was stated that this was done to ensure that the maximum possible concentration of the test substance in the receptor fluid did not exceed 10% of the determined maximum solubility. The actual dose applied to each membrane was determined by counting an aliquot of the dosing solution, and calculating the amount of the test compound from the total radioactivity applied and the specific activity of the dosing solution.

   The radiochemical purity of the test substance in isopropyl myristate was 95.84%, with a concentration of 88.9 mg/mL and a specific activity of 0.2988 µCi/mg. The maximum solubility of the test substance in the receptor fluid was approximately 1630 µg/mL.

2. **Skin preparation:** Samples of full thickness human cadaver abdominal skin were obtained from the National Disease Research Interchange and stored at approximately -20°C until use. Skin samples were removed from three male donors within 24 h of death and used within three months. The samples were thawed at room temperature, immersed in 60°C water for 45 seconds to 2 minutes, and the epidermis was then peeled away from the dermis. The resulting epidermal membranes were then placed on aluminum pans and stored refrigerated (0-10°C) until use. The membrane thicknesses ranged from 43-72 µm.

3. **Membrane equilibration, flow cell assembly, and assessment of membrane integrity:** A diagram of a flow cell is presented in Figure 1 at the end of this DER. The membranes were hydrated in 0.9% saline for approximately 15 minutes. Each membrane was mounted on the top of a flow cell receptor chamber (filled with 0.9% saline), *stratum corneum* up. A donor chamber (0.64 cm^2 exposure area) was clamped on top of the membrane and filled with 0.9% saline. The membrane was then allowed to equilibrate for approximately 30 minutes. During this time, the flow cells were heated with a recirculating water bath to yield a receptor fluid temperature of 32±1°C. After the equilibration period, the integrity of each membrane was assessed by measurement of electrical impedance. Membranes with impedances of ≥17 kilohms were considered to be intact. The saline in the donor and receptor chambers was then removed and replaced with receptor fluid, and allowed to equilibrate at least 15 minutes prior to dosing.

4. **Experimental groups**

   a. **Permeability coefficient (Kp):** The test compound was applied at a dose volume of 100 µL/cm^2 to a total of six skin membranes. After dosing, the donor chamber was sealed with Parafilm®. Duplicate receptor fluid samples (50 µL) were taken at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h post-dosing. The volume of receptor fluid removed was replaced with an equal volume of fresh receptor fluid.

   b. **Short-term penetration rates:** The test compound was applied at a dose volume of 30 µL/cm^2 to a total of 12 skin membranes. The exposures were terminated at either 10 or 60 minutes post-dosing, with equal numbers of membranes taken for each time point.

5. **Terminal procedures:** For both groups, the skin surface was washed with a 2% Ivory® soap solution, followed by rinsing with deionized water. The wash/rinse was saved for analysis. The receptor fluid of the permeability coefficient experiment was discarded. The receptor fluid of the short-term absorption rate experiment was retained for analysis. The donor and receptor chambers were refilled with saline, and the electrical impedance of all membranes was measured again. The saline of the donor chamber was removed and retained for analysis; the receptor chamber saline was discarded. The donor chamber was then removed and rinsed with acetonitrile, which was saved for analysis. The skin membranes were removed and retained for analysis.

   The serial receptor fluid samples, skin wash/rinse, and donor chamber rinse were analyzed directly for radioactivity by LSC. The skin membranes were digested with Soluene®-350 at 60°C with constant shaking (time not provided) and then analyzed for radioactivity by LSC.

The limit of detection of each sample was defined as twice the background rate for appropriate blank samples.

6. **Calculations:** The steady-state penetration rate was determined by plotting the cumulative amounts of µg equivalents of the test compound detected in the receptor chamber at each time point, adjusted for total receptor fluid volume, against time (h) to yield an absorption profile. The Kp (cm/h) was calculated by dividing the penetration rate (defined as the slope of the line at steady-state [µg equivalents/cm^2/h; at least four data points]) by the concentration of the test compound (µg/cm^3). The short-term absorption rate (µg equivalents/cm^2/h) for each exposure interval (10 or 60 minutes) was calculated by dividing the sum of the µg equivalents of test material in the receptor fluid and skin by the skin exposure area and exposure time. Total recovery of radioactivity was defined as the sum of the radioactivity in the receptor fluid, washed/rinsed from the skin and donor chamber, and in/on the skin membrane. Data were presented as mean (±SD); statistical analyses were not performed.

II. RESULTS

A. **PERMEABILITY COEFFICIENT:** Results of the permeability coefficient experiment are presented in Tables 1a and b. The integrity of the skin membranes was not affected by exposure to the test substance, as the average ratio of electrical impedance measured after and prior to dosing was 1.40. The test substance penetrated the skin readily, as it was detectable in the receptor fluid at 0.5 h post-dosing (Table 1a). The amount absorbed appeared to increase in a linear fashion, suggesting steady-state penetration had been achieved. The steady-state penetration determined from a minimum of four data points was 12.3 µg equivalents/cm^2/h. After 8 h, 1.02% of the applied test compound was detected in the receptor fluid, demonstrating that the receptor fluid offered sink conditions to the test compound. The permeability coefficient (Kp) was calculated to be $1.38x10^{-4}$ cm/h.

TABLE 1a. Mean (±SD) cumulative absorption (µg equivalents/cm^2) of naphthalene through human skin membranes.[a]

Time (h)	Absorption
0.5	6.25
1	11.4±3.87
2	24.4±11.3
3	37.0±19.4
4	49.5±24.6
5	60.7±32.7
6	71.4±38.5
7	84.3±46.9
8	97.6±55.3
Penetration rate (µg equivalents/cm^2/h)	12.3±6.68
% absorbed at 8 h	1.02±0.58
Permeability coefficient (cm/h)	$1.38x10^{-4}$±$7.51±10^{-5}$

a Data were obtained from Tables 2 and 3 on pages 22-23 of the study report.

Total recovery of the applied dose in the permeability coefficient experiment was 96.8% (Table 1b).

TABLE 1b. Mean (±SD) recovery (% of applied dose) of naphthalene applied to human skin membranes.[a]

Compartment	Recovery
Receptor fluid	1.02±0.58
Skin wash	95.1±2.58
Skin membrane	0.67±0.34
Donor chamber wash	0.056±0.055
Total recovery	**96.8±2.06**

a Data were obtained from Table 4 on page 24 of the study report.

B. SHORT-TERM PENETRATION RATES: Results of the short-term penetration experiments are presented in Tables 2a and b. The integrity of the skin membranes was not affected by exposure to the test substance, as the average ratio of electrical impedance measured after and prior to dosing was 0.96 and 0.86 for the 10 and 60 minute exposures, respectively. Following a 10 minute exposure, 0.8 µg equivalents of the test compound were found in the receptor fluid and 14.8 µg equivalents were found in the skin (Table 2a). From these data, a penetration rate of 142.6 µg equivalents/cm^2/h was calculated. Following a 60 minute exposure, 4.9 µg equivalents of the test compound were found in the receptor fluid and 11.1 µg equivalents were found in the skin. From these data, a penetration rate of 25.0 µg equivalents/cm^2/h was calculated.

TABLE 2a. Mean (±SD) short-term absorption (µg equivalents) and penetration rates (µg equivalents/cm^2/h) of naphthalene through human skin membranes.[a]

Compartment	Mean±SD
10 minute exposure	
Receptor fluid	0.83±0.83
Skin	14.8±15.0
Total absorbed	15.5±15.1
Penetration rate	142.6±139.1
60 minute exposure	
Receptor fluid	4.89±2.97
Skin	11.1±7.59
Total absorbed	16.0±9.76
Penetration rate	25.0±15.3

a Data were obtained from Table 6 on page 26 of the study report.

Total recoveries of the applied doses in the short-term penetration rate experiments were 94.8% and 93.5% for the 10 and 60 minute exposures, respectively (Table 2b).

TABLE 2b. Mean (±SD) recovery (% of applied dose) of naphthalene applied to human skin membranes.[a]

Compartment	Recovery
10 minute exposure	
Receptor fluid	0.044±0.044
Skin wash	93.9±2.51
Skin membrane	0.78±0.79
Donor chamber wash	0.13±0.09
Total recovery	**94.8±1.75**
60 minute exposure	
Receptor fluid	0.26±0.16
Skin wash	92.6±3.29
Skin membrane	0.58±0.40
Donor chamber wash	0.11±0.14
Total recovery	**93.5±3.24**

a Data were obtained from Table 7 on page 27 of the study report.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS= CONCLUSIONS:** Based on the slope at steady-state (12.3 µg equivalents/cm^2/h) and the concentration of the applied dose of naphthalene (88,876 µg/cm), the permeability coefficient was calculated to be 1.38x10^{-4} cm/h. Based on the amount of naphthalene in the receptor fluid and skin, an exposure area of 0.64 cm^2, and an exposure time of 10 minutes, the short-term penetration rate was calculated to be 142.6 µg equivalents/cm^2/h. Following a 60 minute exposure, the short-term penetration rate was calculated to be 25.0 µg equivalents/cm^2/h.

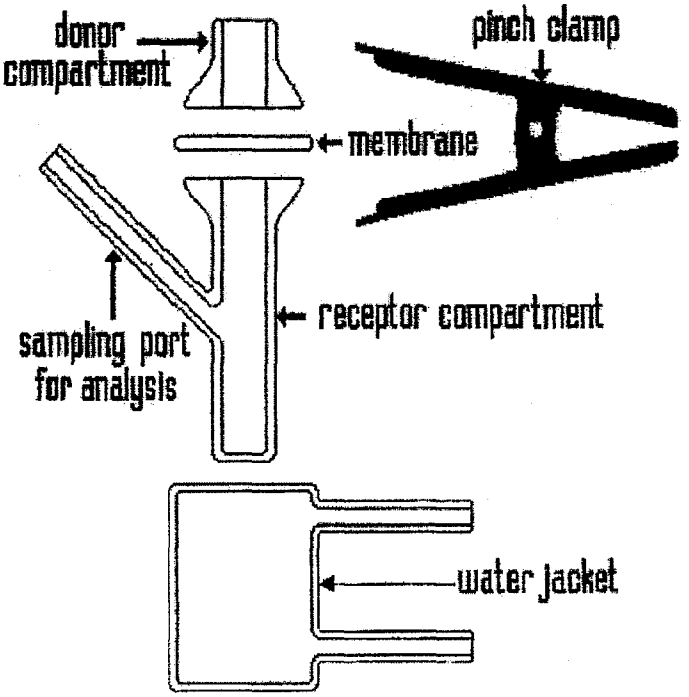
B. **REVIEWER COMMENTS:** The integrity of the skin membranes was not affected by exposure to the test substance. The test substance penetrated the skin readily, and the amount absorbed appeared to increase in a linear fashion, suggesting steady-state penetration had been achieved. The steady-state penetration determined from a minimum of four data points was 12.3 µg equivalents/cm^2/h. After 8 h, 1.02% of the applied test compound was detected in the receptor fluid, demonstrating that the receptor fluid offered sink conditions to the test compound. From these data, the permeability coefficient was calculated to be 1.38x10^{-4} cm/h. Following a 10 minute exposure, 0.8 µg equivalents of the test compound were found in the receptor fluid and 14.8 µg equivalents were found in the skin. From these data, a penetration rate of 142.6 µg equivalents/cm^2/h was calculated. Following a 60 minute exposure, 4.9 µg equivalents of the test compound were found in the receptor fluid and 11.1 µg equivalents were found in the skin. From these data, a penetration rate of 25.0 µg equivalents/cm^2/h was calculated.

This study is **acceptable/non-guideline.**

C. **STUDY DEFICIENCIES:** No deficiencies were observed.

Figure 1.

Figure 1: Static diffusion cell



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R172658

Chemical Name: 2-Amino-2-methyl-1-propanol

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HED File Code: 258 Contractor DER TOX Caution: This Document (review) was completed by a contractor and has not undergone secondary review. This document may not reflect Agency policies.
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